Effect of Nitrogen and Cytokinins on Photosynthesis and Growth of sorghum (*Sorghum bicolor* (L.) Moench) under *Striga hermonthica* infestation.

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A Thesis Submitted in Partial Fulfilment of the Requirement for the degree of Master of Science in Crop Science (Protection) at Wageningen Agricultural University, The Netherlands

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January, 1998

ACKNOWLEDGEMENT

This study has been guided and supported by many persons to whom I would like to express my gratitude.

I would like to express my sincere appreciation to Ing. Aad van Ast who inspired me to carry out this study. His unrelenting assistance throughout the period of my study provided me with the much needed confidence.

I would like to convey my gratitude to Prof. Dr Ir Jan Goudriaan for accepting to supervise this piece of work. His constructive criticisms were invaluable.

I am greatly indebted to Dr Wilco Jordi and Ing. Geert Stoopen of AB-DLO for their contribution on plant hormones.

Many thanks to Taede Stoker and colleagues, Ing. Peter van Leeuwen, Ans Hofman, Ing. Jacques Withagen, Esther Tempel and Slavica Ivanovic for their assistance in some analyses and measurements.

My special thanks go to Dr Kees Eveleens who made my dream of studying in Wageningen come true.

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SUMMARY

Striga spp. are obligate hemi-parasites belonging to the plant family *Scrophulariaceae*. They attack the roots of host plants including a number of widely grown tropical cereal (sorghum, millets, maize, rice) and legume (cowpea, groundnut, sweet potato) crops. *Striga hermonthica* is the largest of the species occurring commonly as weeds. It attacks only grasses and its damage to cereal crops often results in significant yield losses.

Host photosynthesis is one of the processes severely affected by *Striga hermonthica* infection. Significantly lower assimilation rates as well as light use efficiencies have been measured in *Striga*-infected hosts. Additions of nitrogen have been found to correlate positively with assimilation rate in *Striga*-infected hosts. Some cultivars that are tolerant of *Striga* infection have been found to have similar assimilation rates and as in the uninfected. However, there is still limited information on the primary mechanism(s) by which host photosynthesis is affected and on the role of nitrogen mediating these effects. The observed effect on photosynthesis has been correlated with stomatal limitations. Observed changes in host hormone balance have also been linked to some of the effects including assimilation. Far less cytokinins and gibberellins have been measured in *Striga*-infected host compared with the uninfected as opposed to excessively high amounts of absiscic acid.

In an attempt to explain the effects of *Striga hermonthica* on host photosynthesis and the possible role of nitrogen and hormone balance, a greenhouse (pot) experiment was conducted on two sorghum cultivars: the highly *Striga*-sensitive CK60B and the more tolerant land race "Tiemarifing". The two cultivars were grown in the presence or absence of *Striga hermonthica*, with or without a single addition of nitrogen fertiliser (the equivalent of 60 kg/ha) and with or without cytokinin (zeatin, 10^{-4} M) applications. Cytokinins have been found to be the most limiting in *Striga*-infected host and it was hypothesised that exogenous application could disrupt the imbalance and possibly reverse the negative effect of *Striga* on sorghum photosynthesis.

For one reason or the other, the cultivar "Tiemarifing" appeared to have lost its status of tolerance in the greenhouse. This cultivar performed poorly and no conclusive results could be obtained.

On the contrary, significantly higher assimilation rates were measured at 72 days after emergence (DAE) of sorghum in *Striga*-infected CK60B with cytokinin application irrespective of nitrogen. This effect was similar for the computed stomatal conductance, transpiration rates and leaf chlorophyll content. Amax values were also higher in those treatments in comparison with the controls even though the light use efficiencies were lower. Treatments receiving both nitrogen and cytokinins in the absence of *Striga* had significantly lower assimilation rates, stomatal conductance, transpiration as well as chlorophyll content of the leaves.

The apparent increase in assimilation of *Striga*-infected CK60B did not, however, lead to a corresponding increase in the total biomass measured at time of harvest, 72, DAE. The infected plants were still significantly shorter over time than the noninfected irrespective of cytokinin applications or nitrogen. The dry matter partitioning was severely affected by *Striga* infection. Internode expansion was virtually inhibited whereas leaf number or root dry weight was hardly affected resulting in higher root:shoot and leaf area ratios in the *Striga*-infected treatments. Nitrogen application resulted in an increase in the assimilation rate of *Striga*-infected plants as well in a significant delay in the time of *Striga* emergence.

Results indicate that the increase in assimilation rate of *Striga*-infected sorghum following addition of nitrogen might be mediated by cytokinins. The decrease in total biomass of *Striga*-infected plants irrespective of cytokinin or nitrogen despite an increase in assimilation rate when the latter two are applied, suggests possible toxic effect(s) of *Striga* on sorghum. The almost complete inhibition of internode expansion is an indication that the hormone balance might be the most disrupted. More information is needed to fully understand the mechanism.

1. INTRODUCTION

1.1 The *Striga* problem

Striga spp. are obligate hemi-parasites belonging to the plant family *Scrophulariaceae*. They attack the roots of host plants including a number of widely grown tropical cereal and legume crops. *Striga hermonthica* is believed to be the most important parasitic weed species on a world scale (Parker and Riches, 1993).

Damage by *Striga* spp. often results in significant yield losses. In Africa, estimated cereal grain losses associated with *Striga* damage are about 40% when averaged across the whole continent (Lagoke *et al.*, 1991). Losses of 100% are, however, not uncommon under heavy infestation. *Striga* may have already become the greatest biological constraint on food production in Africa, a more serious problem than insects, birds or plant diseases (Ejeta *et al.*, 1992). In India, some 25,000 tons of sorghum grain are lost due to damage by *Striga* annually in the State of Andhra Pradesh alone (Doggett, 1988).

Traditional cropping practices of crop rotations and long fallows kept *Striga* bounded in the past. With an ever increasing human population in most of the *Striga*-stricken regions, those practices have virtually been abandoned in favour of continuous cropping of host crops to meet the food demands. This practice has worsened the *Striga* problem as it offers a conducive environment for *Striga* spp. to flourish.

1.2 *Striga hermonthica*: Origin and distribution

Striga hermonthica, the most important weed species on a world scale (Parker and Riches, 1993), parasites cereal food crops (sorghum, pearl millet, maize, upland rice, finger millet) and a wide range of wild grasses, including *Setaria incrassata, Sorghum arundinaceum, Roettboelia cochinchinensis, Eleusine indica, Paspalum scrobiculatum, Aristida, Bracharia, Cymbopogon* and *Digitaria* species (Dembélé *et al.*, 1994; Farah, 1991; Kenfack *et al.*, 1996; Musselman & Hepper, 1986; Parker & Riches, 1993; E. Kuiper, personal observation in Kenya, 1993, Burkina faso and Mali, 1994, Ethiopia, 1996) in fallow lands and field bunds. It is suggested that *Striga hermonthica* originates from the Sudano-Ethiopian region where it co-evolved with wild relatives of sorghum (Mohamed, 1994; Mohamed *et al.*, 1996; Rao & Musselman, 1987). Following the domestication of sorghum, the "wild" *S. hermonthica* evolved to attack this crop, and subsequently it spread over the continent. *S. hermonthica* probably reached other parts of the continent when sorghum seeds from the Sudano-Ethiopian region were introduced there. The hypothesis of a *S. hermonthica*-sorghum co-evolution is based on the fact that sorghum is one of the few crops that possesses resistance mechanisms against this parasite.

S. hermonthica is distributed throughout northern tropical Africa, extending from the semi-arid areas of Ethiopia and Sudan to the moist savannah areas of West Africa and the Lake Victoria Basin in the east. To the north it extends into south-western Arabia, and to the south into Angola and Namibia. *S. hermonthica* is

considered to be a tropical parasite but in Ethiopia it can be found at altitudes up to 2500m (Kuiper, Reda & Verkleij, personal observations). At such places a more temperate to Mediterranean climate occurs, with occasional night frosts. This suggests that at least in Ethiopia *S. hermonthica* is adapted to lower temperatures, and this could mean that *S. hermonthica* is capable of survival in some parts of Europe too. Research on the temperature limits of these Ethiopian *S. hermonthica* populations is necessary to determine whether Europe and other parts of the world with no *Striga* problems are at risk of an invasion in future.

1.3 Morphology and biology

S. hermonthica plants are erect hairy herbs with robust, quadrangular, fibrous stems. They usually grow 30-40 cm tall and sometimes 100 cm or more. The leaves are linear-lanceolate to lanceolate, with a length of 2.5-7.5 cm and up to 2 cm wide. Flowers are 1-2 cm across, placed alternate in a dense spike. The spikes of 6-10 open flowers can be very striking. Spikes containing more than 10 flowers open are an indication of impaired pollination. Flowers are usually bright pink with distinctly five-ribbed calyx, corolla tube 11-17 mm in length, bending characteristically at an angle immediately over the tip of the calyx, but they may also vary in colour from rose-red to bright pink, and occasionally completely white flowers are observed (Doggett, 1988; Mohamed, 1994; Musselman & Hepper, 1986; Pieterse & Verkleij, 1991; Raynales-Roques, 1987). In general, there is a lot of variability in most morphological characters (Mohamed, 1994).

S. hermonthica has a very complex life cycle, which is completely adapted to that of its host. Starting with the seeds, *S. hermonthica* produces dust-like seeds in capsules, each seed measuring about 0.3 mm in length , 0.2 mm in width, and weighing about 7 µg. The seeds have a characteristic surface pattern of ridges (Musselman and Parker, 1981) which is distinctive and uniform within a single plant of *S. hermonthica* (Jones and Safa, 1982) but varies considerably from plant to plant. The number of seeds per capsule is about 700 and on average 60-70 capsules may be produced by a single *S. hermonthica* plant. Hence numbers over 50,000 seeds can certainly occur in well-grown *S. hermonthica* (Parker & Riches, 1993). Observations by a number of authors suggest that seeds of *S. hermonthica* can survive 20 years in the field, but real data to back this claim are scarce. Seeds of *S. hermonthica* on shedding are not able to germinate. They enter primary dormancy which in most parasitic plant species is due to immature embryos (Mayer & Poljakoff-Mayber, 1963). Little is known about the period required for embryos to reach maturity. Parker (1984) observed that, in general, seeds of *S. hermonthica* show a low germination percentage during the first six months after collection. Genetic differences between populations may play a role in primary dormancy (Pieterse and Verkleij, 1994) resulting in variations with seeds collected from different regions.

The seeds of *S. hermonthica*, even after primary dormancy is broken, are not able to germinate until they have been imbibed (conditioned) for at least a few days and ideally for 1-2 weeks, at a temperature of between 25 and 30°C under moist conditions, for optimal germination (Brown, 1965; Okonkwo, 1991; Ransom

& Njoroge, 1991; Reid and Parker, 1979; Vallance, 1950). Higher temperatures will result in more rapid conditioning of *S. hermonthica* seeds but percentage germination will not be as high even after several weeks (Vallance, 1950; Reid and Parker, 1979). There is evidence that prolonged conditioning beyond a few weeks, especially at higher temperatures, will not result in secondary or wet dormancy (Vallance, 1950; Reid and Parker, 1979; Pieterse *et al.*, 1984). Germination after conditioning is only possible when the seeds are exposed to a stimulant excreted by the roots of the host plant. Conditioned seeds exposed to stimulant under suitable temperature and moisture conditions will germinate within 24 hours. The earliest detectable response is production of ethylene, while the first visible response, protrusion of a small radicle through the seed coat, is detectable within 10 hours (Thuring *et al.*, 1994).

Once the seeds of *S. hermonthica* have germinated, up to several millimetres from a host root, they still have to make contact with the root in order to parasitize it. It has been observed that radicle growth of *S. hermonthica* is directed towards the host root through chemotropism i.e. chemical compounds in root exudates excreted by the host (Williams, 1961). When the radicle reaches the host root, cell elongation stops abruptly and the development of the haustorium begins. This process has been shown to depend on haustorium-initiating substances. Kuiper (1997) summarises the major features of haustorial formation as follows:

-Rapid cessation of cell elongation

-A shift to radial expansion of cells in the cortical position of the radicular tip. This reorientation of cell enlargement leads to formation of a little bulb which is the first detectable sign of haustorium formation.

-The initiation of haustorial hairs. Hairs are visible within 10-12 hours after the onset of haustorium formation. These hairs are different from normal root hairs in that they develop a specialised surface coating, important for the attachment process.

-The development of a group of cells containing many vesicles at the apex of the haustorium. The role of these cells is still not understood.

The switch to haustorial formation must be made within four days after germination or the seeds will lose that capacity because of exhaustion of its resources (Boon *et al.*, 1995; Riopel & Timko, 1995). After attachment, the actual penetration of the host tissue occurs by development of intrusive cells at the root tip which penetrate the cortex of the host root, apparently producing enzymatic secretions so that host cells are separated rather than intracellularly-penetrated (Olivier *et al.*, 1991; Riopel & Timko, 1995). The next step of penetration is the invasion of the host vascular system. At this stage some of the intrusive cells in the central zone of the haustorium have already differentiated into xylem cells, resulting in a functional xylem just above the front of the intrusion zone. When the endodermal barrier is breached, the haustorial cells advance to the host vessels, entering the lumen of especially the large vessels through the side-wall pits or by dissolution of the vessel wall (Dörr, 1996; Riopel & Timko, 1995). There is rapid development of direct links between parasite and host xylem systems

once the haustorium is inside the host stele. Phloem connections are not formed. Soon after the establishment of the first haustorium, adventitious secondary roots arise from the primary *Striga* root. Where these roots make contact with other young host roots still possessing root hairs, secondary haustoria are formed, providing the parasite with further points of contact with the host.
While still below ground *Striga hermonthica* depends totally on the host for its organic nutrition since it only forms root hairs where it touches the host root. Once the *Striga* plants have emerged above the ground, they develop functional chlorophyll, but there is a continued movement of carbohydrates, minerals, and water from the host to parasite. Where there are favourable conditions for parasite growth, *S. hermonthica* will normally emerge after 4-5 weeks following host germination and flower within 7-8 weeks. Viable seeds are probably produced within 2 weeks of the flower opening and being pollinated, but are not fully matured and shed until about 2 weeks later (Parker & Riches, 1993).

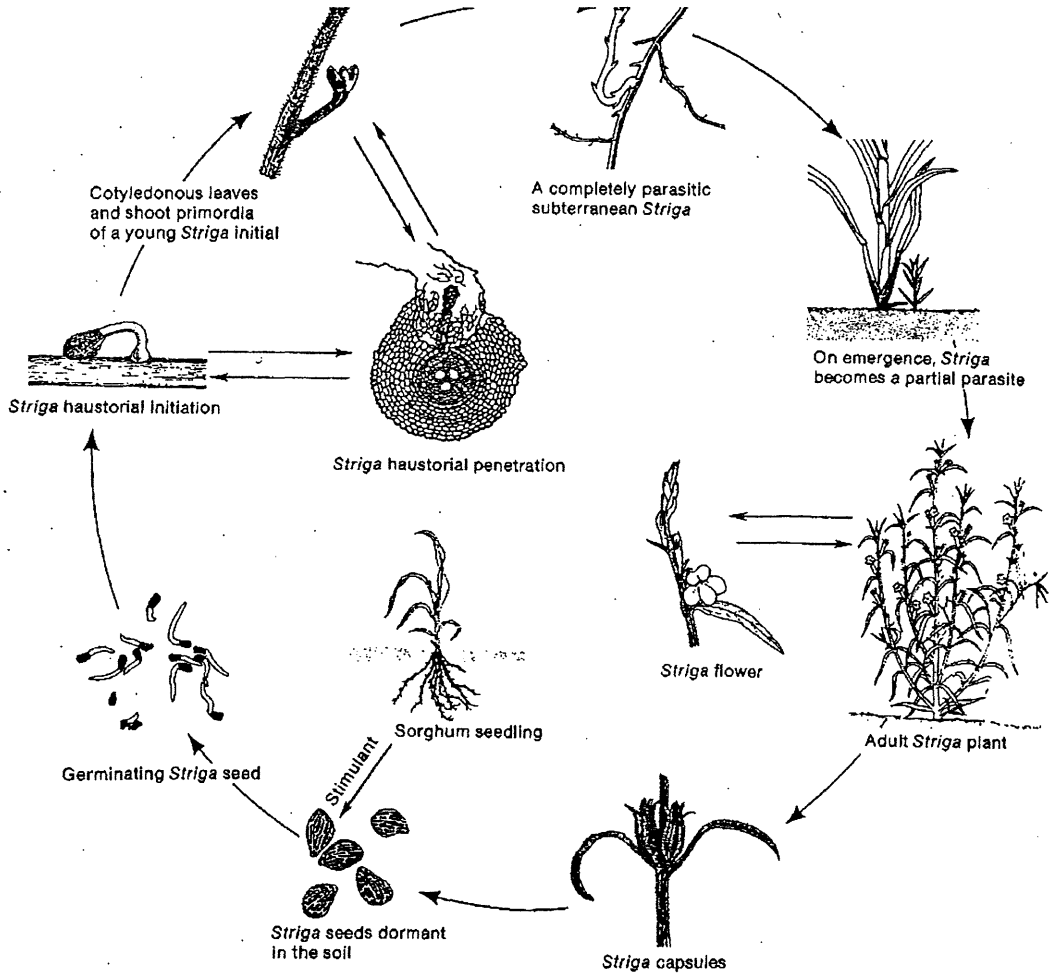

Figure1.1 Life cycle of *Striga hermonthica* (courtesy of International Crops Research Institute for the Semi-arid Tropics)

1.4 Effects of *Striga hermonthica* on sorghum (host)

Striga hermonthica causes stunting of the host shoot and failure of panicle formation (Ramaiah *et al.*, 1983). Other symptoms include wilting and leaf chlorosis (Doggett, 1988; Parker, 1991). Furthermore, internode expansion and leaf area are often decreased, total host biomass can be strongly reduced, and a spectacular change in dry matter allocation is often observed in infected hosts with consequent increase in root:shoot ratio (Cechin and Press, 1993; Graves *et al.*, 1990; Parker and Riches 1993; Press and Graves, 1991). The change in root:shoot balance and the resultant reduction in host shoot growth can occur at a very early stage of parasite growth even before it emerges from the soil (Parker & Riches,1993; Press *et al.*, 1996). The effects are partly a consequence of the withdrawal of carbon, mineral nutrients and water from the host (Press & Whittaker, 1993). The reduction of host growth can be enormously greater than is explained by the simple removal of resources by the parasite. When *Striga hermonthica* on sorghum plants were only a few millimetres long, it was estimated that the reduction in host weight was at least 30 times the weight of the parasite (Parker, 1984). The production of toxins by the parasite has been suggested, but the precise nature of this toxin or its effect is still not fully understood (Doggett, 1988; Parker & Riches, 1993). Interference of the parasite with the host's hormone balance is also regarded as the most likely explanation of *Striga* damage (Drennan & El Hiweris, 1979; Frost *et al.*, 1997; Taylor *et al.*, 1996). Competition for nutrients must also be a potent factor (Doggett, 1988). The good yield responses obtained by applying nitrogenous fertiliser suggest that competition for nitrogen is of major importance (Doggett, 1965; Drennan & El Hiweris, 1979; Ramaiah & Parker 1982).

Apart from the damage to the host shoot system, there is now evidence of a reduction of the photosynthetic efficiency in the host due to *S. hermonthica*. Photosynthetic rates in sorghum can be less than 50% of those measured in uninfected plants (Cechin and Press, 1993 a,b; Frost *et al.*, 1997; Graves *et al.*, 1989; Gurney *et al.*, 1995; Press and Stewart, 1987; Press *et al.*, 1987; Ramlan and Graves, 1996). It has been suggested that the reduction of host photosynthesis accounts for more than 80% of the carbon loss by the host (Press & Graves, 1991).

Another effect of the parasite is its ability to induce water stress in the host, which under heavy infestations occurs even under conditions of normal water supply to the host (Ramaiah *et al.*, 1983). This becomes especially apparent when the parasite emerges, but sometimes water stress is observed before emergence.

1.5 Theoretical background and objectives of the study

1.5.1 *Striga hermonthica*, nitrogen and host assimilation

Host photosynthesis is one of the key processes affected by *Striga* infection. Significantly lower rates of photosynthesis (Press *et al.*, 1987b; Smith *et al.*,

1995) as well as low light use efficiencies (Stewart *et al.*, 1991; Teklu, 1995; Ramlan and Graves, 1996) have been measured in *Striga*-infected sorghum as compared with the uninfected. Carbon budget models suggest that lower rates of CO_2 fixation by the host canopy may account for as much as 80% of the difference in productivity between infected and uninfected plants (Graves *et al.*, 1989; Graves *et al.*, 1990). In recent studies on the effect of *S. hermonthica* on the growth and photosynthesis of sorghum (susceptible commercial varieties CK60/CSH-1 and the local land race "Ochuti") by Gurney *et al.* (1995) in the field, and Frost *et al.* (1997) in root observation chambers, the rate of steady-state photosynthesis of the youngest leaf of *Striga*-infected "Ochuti" was comparable with that of uninfected plants throughout most of the time course of the association whereas significantly lower rates were observed on the leaves of the *Striga*-infected commercial varieties compared with control plants. Similar results have been observed by van Ast (1998a,b) on the local land race, "Tiemarifing", and the commercial variety CK60B. Even though Cechin and Press (1993a) found a positive linear correlation between photosynthesis and the concentration of nitrogen supplied to *Striga*-infected and noninfected sorghum plants, there is still limited information on the primary mechanism(s) by which host photosynthesis is affected by *Striga*, and on the role of nitrogen mediating these effects.

The extractable activity of key enzymes of photosynthetic metabolism, RUBISCO, PEP carboxylase and NADP malic enzyme (Press and Cechin, 1994; Smith *et al.*, 1995), and the chlorophyll content and amount of soluble carbohydrates or starch of leaves (Frost *et al.*, 1997), do not appear to be altered by *Striga* infection. The reduction of photosynthesis thus appears to be a consequence of both stomatal and biochemical limitations (Frost *et al.*, 1997; Press *et al.*, 1996; Ramlan & Graves, 1996; Smith *et al.*, 1995).

The synthesis of the key enzymes of photosynthetic metabolism, RUBISCO, PEP carboxylase and NADP malic enzyme could be perturbed by *Striga* infection resulting in limited amounts in the leaf. Relatively lower amounts of RUBISCO and PEP carboxylase were measured in *Striga*-infected CK60B and "Tiemarifing" sorghum plants compared with the controls (van Ast, 1998a). It has been observed in some C_3 plants that, as a wounding response, the expression of genes of the small subunit of the ribulose biphosphate carboxylase is turned off (Peña-Cortés *et al.*, 1988). *Striga* attachment and penetration into the host root could have such a wounding effect with the host responding in a similar manner resulting in negative effects on photosynthesis. Also, according to Stuart Chapin III (1991) a low nitrogen supply results in a low concentration of photosynthetic enzymes, which in turn causes a low rate of photosynthesis per gram of leaf. Besides, nitrogen stress also causes a decline in stomatal conductance that could also explain the decline in photosynthesis. Carbon allocation models have shown that nitrogen deprivation leads to reduced cytokinin production, a decreased rate of cytokinin export from the roots to the shoot, and decreased cytokinin concentrations (Van der Werf and Nagel, 1996). If the observed effects of nitrogen on physiology of C_3 plants could apply for C_4 plants like sorghum then

this could partly explain the positive effects of nitrogen application observed in *Striga*-infected sorghum plants.
Sink effects and the reduction of host photosynthesis cannot all explain the effects of *Striga hermonthica* on host growth, especially because most of the effects become visible shortly after attachment of the parasites to the host root system, a stage at which the parasites are still very small (Press *et al.*, 1996). Frost *et al.* (1997) observed that the parasite affected the height of sorghum (CSH-1)-infected plants as early as 4 days after attachment of *Striga* to the roots, before there was a measurable change in the rate of photosynthesis. The production of specific toxins that alter the metabolism of the host has been suggested (Musselman, 1980; Parker, 1984) although data supporting this possibility are still lacking.

1.5.2 *Striga hermonthica* and hormone balance

Striga-infection has an influence on the hormonal balance of the host (Drennan & El Hiweris, 1979; Frost *et al.*, 1997; Taylor *et al.*, 1996). Exceptionally high amounts of ABA were measured in the xylem sap (Drennan & El Hiweris, 1979; Frost *et al.*, 1997) or in the leaves (Frost *et al.*, 1997; Taylor *et al.*, 1996) of *Striga*-infected host plants. In the study by Drennan & El Hiweris (1979) the cytokinins and gibberrellins content of xylem sap from sorghum infected with *S. hermonthica* was lower than in the control plants, suggesting that perturbations in the balance of growth regulators may contribute to changes in host architecture. In the same study, additions of benzyladenine and gibberellins to the root system of infected sorghum plants helped to prevent some of the diminished growth consequent on infection. The data suggested that cytokinin deficiency may be more limiting than the amounts of gibberellins.

1.5.3 Cytokinins and plant growth and development

Cytokinins as plant hormones acting in conjunction or in opposition to other plant hormones, play a major role throughout the development from seed germination to leaf and plant senescence. They modulate physiological processes important throughout the life of the plant including photosynthesis and respiration. They are believed to be actively involved in breaking seed dormancy, de novo bud formation, the release of buds from apical dominance, leaf enlargement, the inhibition of lateral root formation, the shift from vegetative to reproductive development, and delay of senescence. Senescence is delayed by cytokinins through stimulation of the synthesis of chloroplast DNA and photosynthetic enzymes RUBISCO and PEP carboxylases as well as grana formation and chloroplast replication. They also limit stomatal closure (Incoll and Whitelam, 1977; Blackman and Davies, 1983; Incoll and Jewer, 1985) which accompanies senescence thus enhancing transpiration and the flow of nutrients through the xylem. They also inhibit oxidation, preventing the sharp rise in respiration occurring during senescence.

It is remarkable to note that cytokinin-deficiency would lead to symptoms similar to those observed in *Striga*-infected plants. Since ABA and cytokinins act

in opposite directions and may even antagonise each other in some situations (Thimann, 1992), such symptoms would appear when high amounts of ABA are present in the plant, as is the case in *Striga*-infected plants. Cummins *et al.*, (1971) concluded that ABA caused a reduction in photosynthesis as result of stomatal closure, and not because there was a direct action on the photosynthetic capacity of mesophyll chloroplasts. Further studies over the years have appeared to confirm this conclusion (e.g. Frost *et al.*, 1997), but nevertheless some doubts remain and there is a possibility that ABA has a direct action on mesophyll photosynthesis (Raschke & Hendrich, 1985)

1.5.4 Aim and approach of the study

The outcome of great efforts on *Striga* resistance in sorghum over the past decades has so far been disappointing. There are still no sorghum varieties with immunity, or even a high level of resistance reliable over any wide area (Parker and Riches, 1993). This is partly due to distinct morphotypes, physiological strains and races of the species. Some local land races of sorghum such as "Ochuti" grown in western Kenya, "Tiemarifing" in Mali, are reported to show some tolerance to *Striga*. Understanding the mechanism(s) of tolerance in these land races could be of great help in breeding for resistance or tolerance of sorghum to *Striga*.

The primary aim of this study is thus to gain more insight into the effects of *Striga* on photosynthesis and development of its sorghum host and the possible role of cytokinins/nitrogen within this interaction. Blackman and Davies (1983) observed highly significant effects on stomatal opening upon addition of cytokinin solutions to maize leaf fragments, only in the presence of ABA. It is hypothesised that exogenous application of cytokinins to sorghum leaves, in the presence of exceptionally large amounts of ABA consequent on *Striga* infection could help mitigate some of the effects (especially stomatal closure and lower photosynthetic rates) of *Striga* on sorghum by overriding the strong inhibitory effect of ABA. Applied nitrogen may be involved in the synthesis (or reduction of degradation) of carboxylating enzymes as well as endogenous cytokinins among other functions. Following cytokinin/nitrogen applications, photosynthesis and light use efficiency, chlorophyll content, stomatal conductance, cytokinin and ABA levels, and the amount of RUBISCO/PEP carboxylase of the leaves will be measured in two sorghum cultivars: the highly *Striga*-sensitive CK60B and the more tolerant "Tiemarifing".

This is part of a wider study elsewhere using the eco-physiological approach to understand and quantitatively explain the effects of *Striga hermonthica* on sorghum.

2. MATERIALS AND METHODS

2.1 Experimental design

An incomplete (unbalanced) block design was employed using two cultivars of sorghum [*Sorghum bicolor* (L.) Moench.], the highly *Striga*-sensitive inbred cultivar CK60B and a local land race, "Tiemarifing", reported to show some tolerance to *Striga* in Mali. These were grown in the presence or absence of *Striga*, added nitrogen, and with or without exogenous cytokinins (zeatin, trans isomer, Apex Organics Ltd., Devon, UK) application. The design consisted of six blocks, because of the variability of light in the glasshouse, with each treatment replicated five times and each block surrounded by border pots. Since zeatin was dissolved in alcohol (ethanol 96%) and "Tween" (wetting agent), the treatments not receiving any cytokinins were treated with alcohol and "Tween" dissolved in distilled water. To observe whether alcohol/"Tween" had any effect on the treated leaves, four replicates of each treatment were included in the experiment. These received neither zeatin solution nor the alcohol/"Tween".
The two sorghum cultivars were to be compared at a similar physiological stage since they differ in length of the growth cycles as well as photoperiodicity. Unlike CK60B, "Tiemarifing" is photoperiodically sensitive. The comparison was done at the flag leaf (booting) stage. The two were thus harvested at two different dates depending on when most of the noninfected develop flag leaves.

2.2 Plant material and growing conditions

Striga hermonthica (Del.) Benth seeds were collected from infested sorghum fields in Mali (1995). Twenty milligrams of the *Striga* seeds were thoroughly mixed with the top 6 cm soil in 12 litre black plastic pots of the treatments receiving *Striga*, giving an infestation rate of more than 4000 seeds per pot. The percentage germination of the seeds was about 69%. The soil in the pots consisted of a mixture of black soil (Appendix I) and sand in the ratio 1:3 v/v. All pots (infected and noninfected) were placed in the glasshouse with 30/25°C day and night temperatures respectively, during the summer of 1997. The pots were watered regularly for one week to precondition the *Striga* seeds prior to the planting of sorghum. Sorghum seeds collected from Mali (1995), were placed on moistened filter papers in Petri dishes in the growth cabinet to germinate. Two pre-germinated sorghum seeds were planted per pot, and thinned to one per pot one week after emergence. A 13-hour daylength was maintained at the start of the experiment and later reduced to 11 hours, 70 days after emergence (DAE), to induce flowering in the photosensitive "Tiemarifing".
The equivalence of 60 kg/ha of nitrogen, in the form of Urea (0.82g per pot), was added in the pots receiving nitrogen treatment, 10 days after emergence of sorghum. At the same time of application of the nitrogenous fertiliser, 30 kg/ha of phosphorus and potassium in the form of P_2O_5 ("Tri-superphosphate", 0.93 g per pot) and K_2O ("Patent Kali", 0.75 g per pot) respectively, were applied to all treatments to prevent deficiency of these elements.

Trans-zeatin, dissolved in alcohol and "Tween" (as wetting agent) at a concentration of 10^{-4} M, was applied with a sponge to the leaves of plants receiving the cytokinin treatment twice a week. The application started 26 DAE of CK60B when infected plants were already beginning to show chlorotic symptoms and the first shoots of *Striga* emerging. This application was delayed for another two weeks or so (43 DAE) in "Tiemarifing" as symptoms of *Striga* were not apparent even though a few *Striga* shoots had emerged.
Zeatin was applied on the leaves and not soil for at least three reasons:
First, there is evidence that a high proportion of cytokinins occurs in the root (e.g. van Saden and Davey, 1979; Letham, 1978; Godwin *et al.*, 1978). There could be some difficulty with uptake of applied cytokinins by the roots due to differences in concentration gradient especially in the event of passive uptake.
Second, *Striga* infection alters the architecture of the root, and uptake as well as transport of the hormone by the roots could be impaired.
Third, cytokinins can stimulate germination of *Striga* seeds and initiate haustorial development (Babiker *et al.*, 1992). Soil application of this hormone could stimulate germination of *Striga* seeds and initiate haustorial development in the relevant treatments thus affecting the results of the experiment.

2.3 Gas exchange measurements and initial light use efficiency
Before the destructive harvest of plants at 72 and at 98 DAE for CK60B and "Tiemarifing" respectively, the rates of photosynthesis were measured on the youngest fully expanded leaf, that had received a couple of cytokinin applications, to be harvested for the analysis of hormones, enzymes (RUBISCO & PEP carboxylase) and chlorophyll content.
An infrared gas analyser (Model LCA-2 and PLC-N leaf chamber, Analytical Development Co., ADC, Ltd. Hoddesdon, England) was used. Measurements were made halfway along the length of each leaf to be measured. CO_2 at a concentration 350 vpm ($\mu mol.mol^{-1}$) was supplied from a gas cylinder. The surrounding air pressure and temperature in the greenhouse at the time of measurement were 1015 millibars/26 °C respectively. A photosynthetic photon flux density (PPFD) of 1535 $\mu mol.m.^{-2}s^{-1}$ was supplied by two halogen lamps provided with special filters to let through only the spectrum of light between 400-700 nm, photosynthetically active radiation (PAR). The leaf cuvette had an area of 11 cm^2 and an air flow rate of 350 $ml.min^{-1}$ was maintained into the chamber. The software programme *Bladfot5.exe* (Goudriaan and van Kleef, pers. comm.) was used in computing the rates of photosynthesis, transpiration and the stomatal conductance, based on the flow rate and the changes in the CO_2 and water vapour content of the air passing through the leaf chamber.
In order to study the effect of cytokinins/*Striga* on the efficiency of photosynthesis and maximum gross photosynthesis rate at light saturation (Amax), CO_2 uptake of the youngest almost fully developed leaves of the second set of plants from all the treatments was determined. The plants to be measured where placed in the dark and later the leaves were subjected to various photon flux densities from low to high using an updated version of the

assembly described by Louwerse and van Oorschot (1969) and the CO_2 uptake recorded. The procedure is basically the same as with the ADC equipment except for the fact that several photon flux densities are used in the former.

2.4 Chlorophyll content measurements

Four chlorophyll content measurements were taken non-destructively between 29 and 72 DAE using a SPAD-502 chlorophyll Meter (Minolta). Several readings, depending on the size of the leaf, were taken along the whole length of the youngest fully developed leaf, and the values averaged. These readings were not calibrated against the chlorophyll content of leaves. At the time of destructive harvest, 3.14 cm^2 leaf discs were taken from those leaves on which the rates of photosynthesis were determined, and placed in vials containing 5 ml of N,N-Dimethylformamide (DMF) 99%, for extraction of chlorophyll. The absorbance of chlorophyll in DMF was measured at wavelengths 647 and 664.5 nm, with the UV visible recording spectrophotometer (model Shimadzu UV-160A). The total chlorophyll in DMF (milligrams per litre) was determined using the equations of Inskeep and Bloom (1985).

2.5 Carboxylating enzymes and hormone determinations

5 g of leaf samples from leaves on which CO_2 assimilation was measured, were cut off and immediately frozen in liquid nitrogen for the analysis of hormones and photosynthetic enzymes RUBISCO/PEP carboxylase. Proteins were isolated and the concentrations determined as described by Jordi et al. (1996) after extraction by grinding in a precooled mortar under liquid nitrogen. RUBISCO and PEP carboxylase were quantified by densitometric analysis of the protein bands. Plant hormones were determined using the improved enzyme-linked immunosorbent assay (Vonk et al., 1986)

2.6 Growth measurements

Plant height was measured from the base of the stem to the youngest visible ligule at 4 intervals between 19 and 65 DAE. This parameter has previously been shown to be a good correlate of biomass for sorghum (Press and Stewart, 1987). During the destructive harvest 72/98 DAE for CK60B and Tiemarifing respectively, harvested plant material was separated into panicle, stem, leaves and roots. Attached *Striga* counts were made after carefully washing and separating the *Striga* from the host roots. The green leaf area was measured for both sorghum and *Striga* with an area meter (Model LI-3100, Lambda Instruments Corporation, Nebraska, USA). The plant material was oven-dried for 48 hours at 105 °C and the dry weights determined. Counts of emerged *Striga*, relative time of emergence/flowering of *Striga* observations, were made in the course of the experiment so as to be able to eventually explain (partly) some of the effects observed on the host.

Data were subjected to analysis of variance using the statistical package GENSTAT and the treatment means of the variates were compared using Least Significant Difference ($LSD_{0.05}$)

3. RESULTS

The effects of *Striga* appeared to be more serious on "Tiemarifing" than on CK60B later in the course of the experiments. "Tiemarifing", apart from the effects of *Striga*, appeared to be suffering from other biotic/abiotic stresses especially after the first harvest of the CK60B. At the time of first harvest of "Tiemarifing" almost all the *Striga*-infected plants had wilted, especially those in the absence of applied nitrogen. Temperature and light intensity in the greenhouse and the complexity in manipulating the daylength (switching from long to short day) could have played some part in this poor performance. This affected all the subsequent measurements for this cultivar. Only the observations on the sensitive CK60B are reported.

3.1 Growth measurements

The total biomass of the infected was significantly reduced in comparison with the controls. Nitrogen application significantly increased the total biomass (Figure 3.1). There was no significant effect of its interaction with either *Striga* or cytokinin application on the total biomass.

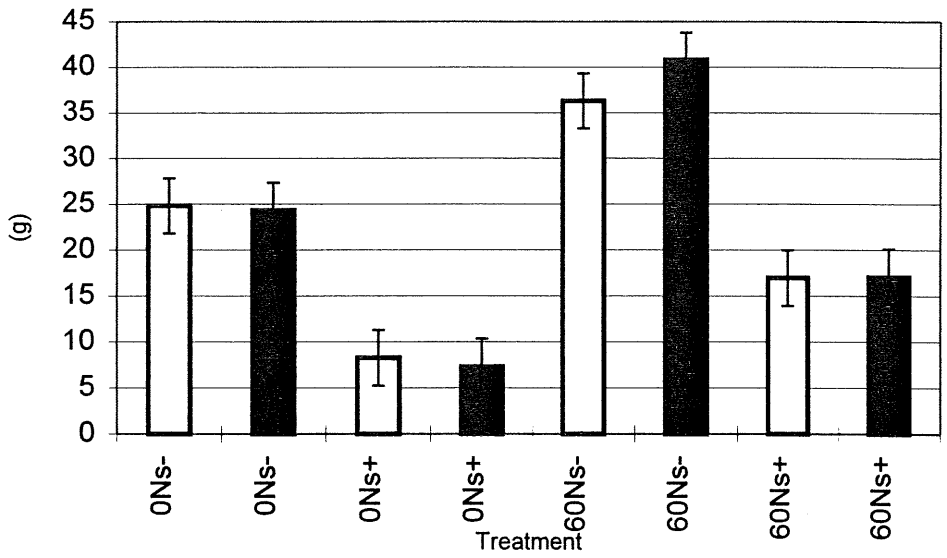

Figure 3.1 Effect of nitrogen, cytokinins and *Striga* (s±) on total biomass (g) of sorghum. ANOVA shows effect nonsignificant (P>0.05). Dark bars = with cytokinin application

The dry matter partitioning was, however, largely influenced in the infected plants. Shoot growth of infected plants was reduced, especially the internode expansion. Root growth was maintained at a level similar to that of uninfected plants or even stimulated resulting in higher root:shoot (Table 3.1) and leaf area (Table 3.2) ratios of infected plants. The two-way interaction between *Striga* and

applied cytokinins also resulted in significantly high leaf area ratios compared with their controls (Table 3.2).

Table 3.1 Effect of *Striga* on the root:shoot ratio and stem dry weight (DW) of sorghum

	root:shoot ratio	DW stem (g)
-	0.2 ± 0.04	12.8 ± 0.9
+	0.7 ± 0.04	2.3 ± 0.9

Mean values ± standard error of 20 replicates are reported.
Analysis of variance shows significant effect of *Striga* (P<.001)

Table 3.2 Effect of *Striga* and cytokinin application on the leaf area ratio ($cm^2.g^{-1}$) of sorghum at harvest 72 DAE

Cyto*	-	+
Striga		
-	37.72	28.54
+	57.50	81.52
LSD(0.05)	11.5	

* cytokinin
The two-factor interaction *Striga**cytokinin is significant (P = 0.021)
Means of 10 replicates are reported.
The least significant difference (LSD) between treatment means is 1.5 (P = 0.05; SE = 6.725, 27 d.f.)

At 19 DAE of sorghum there was already a trend towards reduction in the height of the *Striga*-infected plants compared with the noninfected. All subsequent measurements showed a significant (P<0.001) difference compared with the controls. By 31 DAE *Striga*-infected CK60B plants were 25% shorter than the controls. Before the the destructive harvest of the CK60B, 65 DAE, the infected plants had reached 50% of the height of the controls (Figure 3.2). Nitrogen application did not significantly influence ligule height.

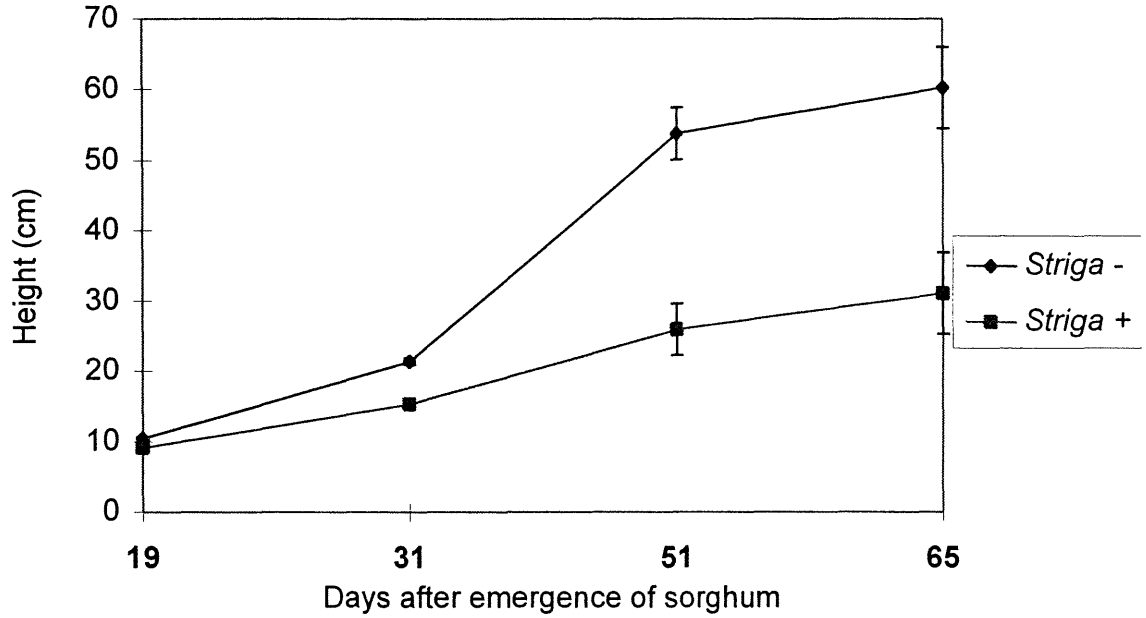

Figure 3.2 Effect of *Striga* on the ligule height (cm) from the base of the stem to the youngest ligule of sorghum. Means and standard errors of 20 measurements are reported.

Table 3.3 Effect of added nitrogen on the relative time of emergence (DAE) of *Striga hermonthica*

Nitrogen	
-	30.18 ± 1.37
+	34.32 ± 1.37

Means of 20 replicates ± standard error are reported
Analysis of variance showed a significant effect ($P < 0.05$)

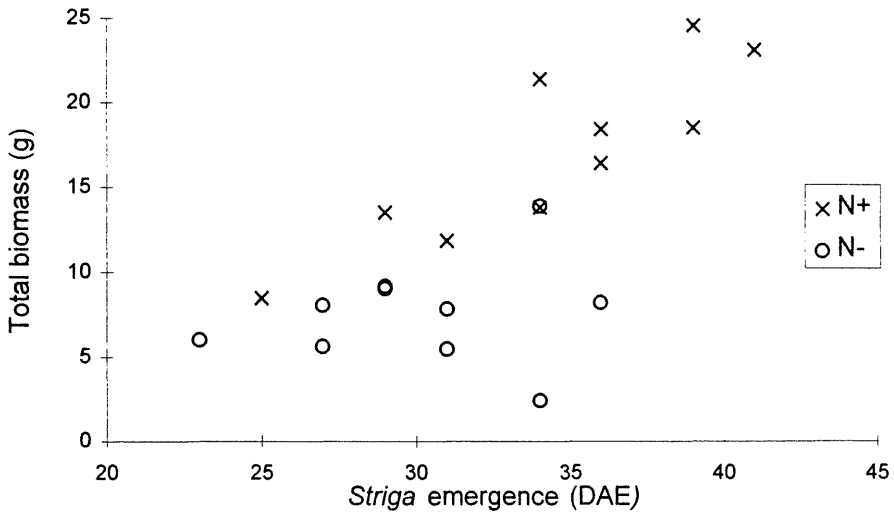

Figure 3.3 Effect of time of emergence first *Striga* shoot on the total dry matter yield of sorghum at time of harvest.

3.2 Gas exchange, stomatal conductance and initial light use efficiency

Treatments receiving cytokinins showed significantly (P = 0.05) higher rates of photosynthesis at the time of measurement 72 DAE (Table 3.4)

Table 3.4 Effect of nitrogen/cytokinin applications and *Striga* on the rate of leaf photosynthesis (μmol $m^{-2}.s^{-1}$) of sorghum 72 DAE at 1535 μmol $m^{-2}.s^{-1}$

		Striga	
		-	+
Nitrogen	Cyto		
-	-	0.35c	0.25c
	+	0.55b	0.43b
+	-	0.70a	0.62a
	+	0.38c	0.75a

The three-factor-interaction nitrogen*cytokinin**Striga* is significant (P = 0.025)
Mean values of 5 replicates are reported.
The least significant difference (LSD) between treatment means is 0.12 (P = 0.05; SE = 0.6895, 27 d.f.)
Different letters indicate significant effects between means in the same column (P = 0.05)

This was only valid for treatments without nitrogen or with nitrogen in the presence of *Striga*. The rates in the infected plants receiving cytokinins were similar to (or even higher than) the noninfected in the absence of applied cytokinins. Significantly lower rates of photosynthesis were measured in the noninfected (without *Striga*) plants receiving both cytokinins and nitrogen applications. Similar results were obtained for the computed stomatal conductance, with a significant (P=0.05) three-factor interaction nitrogen**Striga**cytokinin (Table 3.5). The stomatal conductance and assimilation rate correlated positively for all treatments. The computed rate of transpiration values showed a similar trend even though only the two-factor interactions were significant (Figure 3.5).

Table 3.5 Effect of nitrogen/cytokinin applications and *Striga* on the (leaf) stomatal conductance (mmol m-2. s-1) of sorghum 72 DAE

		Striga	
		-	+
Nitrogen	Cyto		
-	-	4.975b	1.749c
	+	5.098b	2.788c
+	-	7.841a	6.705b
	+	2.929c	8.508a

The three-factor interaction nitrogen*cytokinin**Striga* is significant (P = 0.035)
Mean values of 5 replicates are reported.
The least significant difference (LSD) between treatment means is 1.6 (P = 0.05; SE = 0.923, 27 d.f.).
Different letters indicate significant effects between means in the same column (P = 0.05).

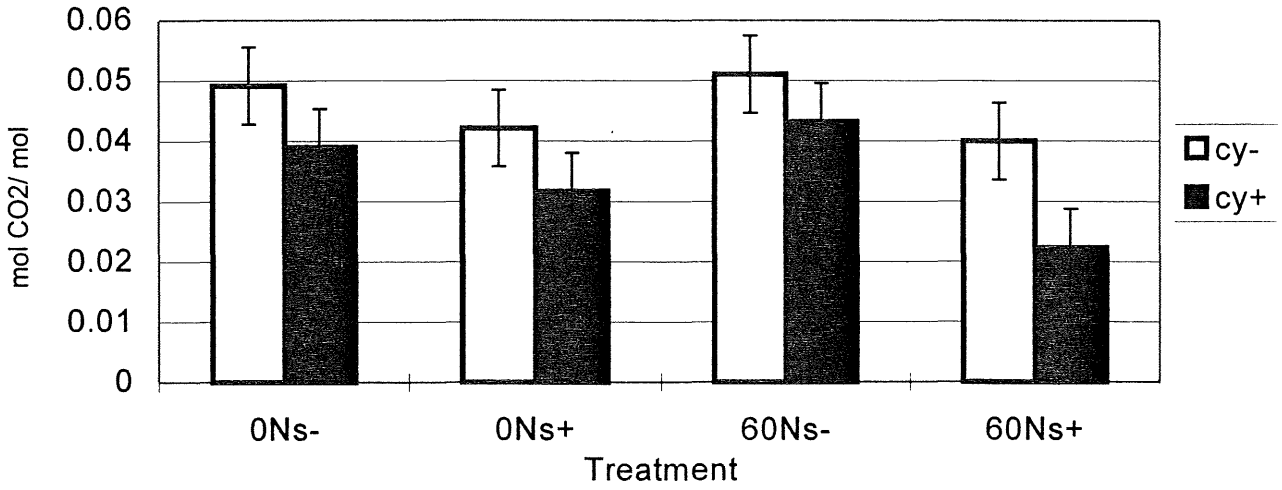

Figure 3.4 Effect of applied cyotokinins (cy±) on the initial light use efficiency of sorghum 10 weeks after emergence. Analysis of variance shows significant effect of cytokinin application (P=0.01).See Figure 3.1 for details.

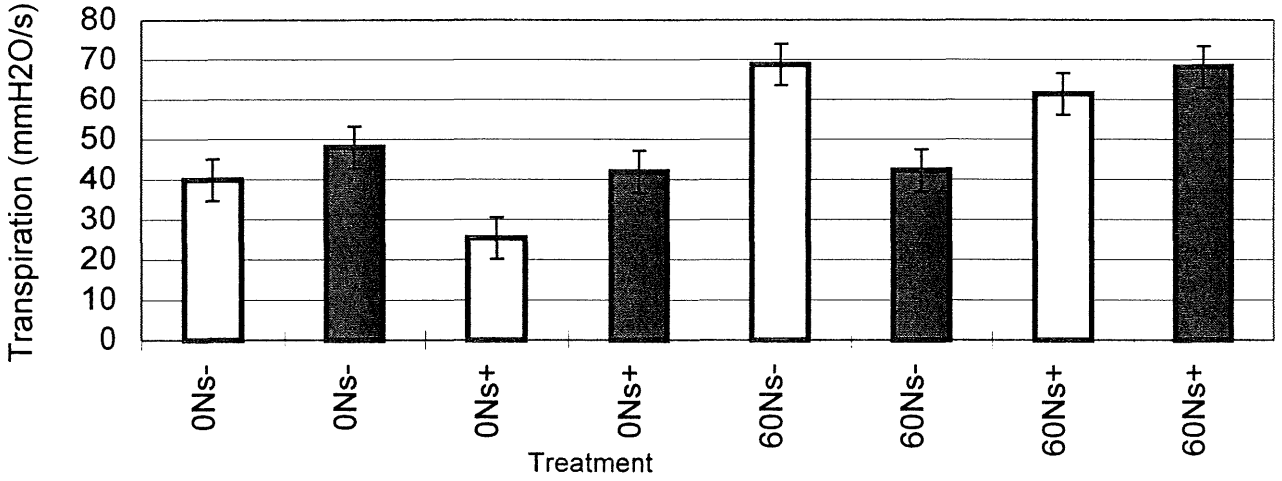

Figure 3.5 Effect of nitrogen, cytokinins and *Striga* on transpiration (mmH2O/s) of sorghum leaf at time of harvest, 72 DAE. Analysis of variance shows only the effect of two-factor interactions significant (P =0.05). See Figure 3.1 for details.

Cytokinin and nitrogen interaction, in the absence of *Striga*, showed a strong negative effect on the stomatal conductance and consequently on the transpiration rate. This negative effect was also observed on the light use efficiency (Figure 3.4) as well as the Amax (Figure 3.6). There was, however, a trend towards decrease light use efficiency in all the treatments receiving cytokinins, and in the presence of *Striga* as well (Figure 3.4).

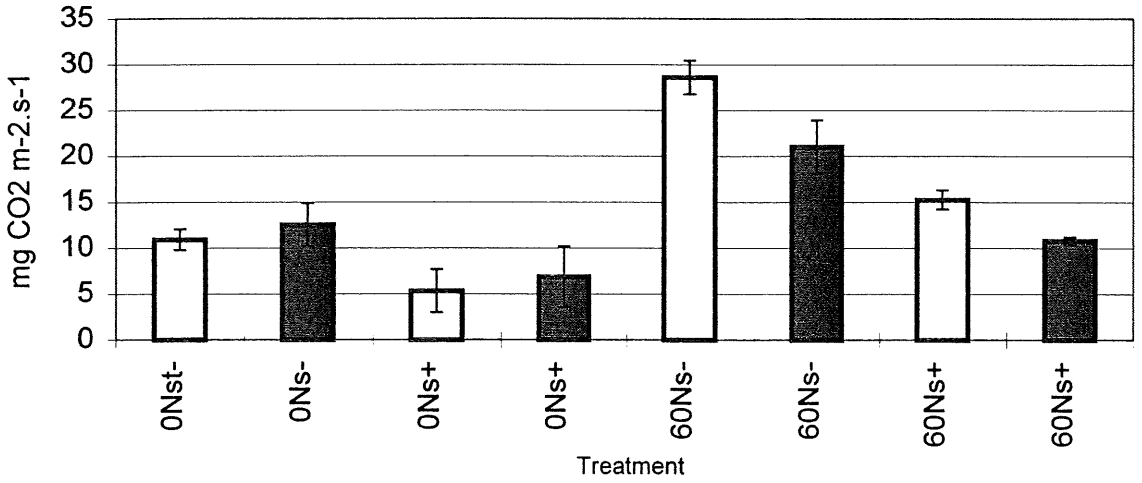

Figure 3.6 Effect of nitrogen, cytokinins and *Striga* (with or without) on the Amax (mg CO2 m-2.s-1) on sorghum leaf 10 weeks after emergence. Analysis of variance shows effect non-significant (P>0.05) See Figure 3.1 for details.

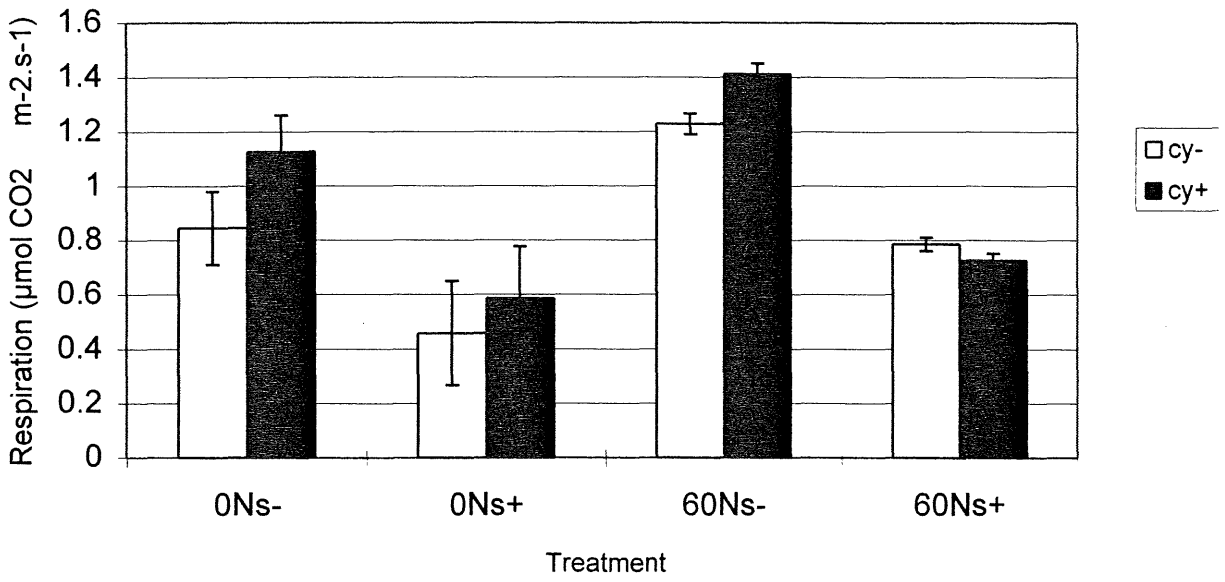

Figure 3.7 Effect of cytokinin (cy±) application on dark respiration (µmol CO2 m-2.s-1) of sorghum leaf. Analysis of variance shows effect non significant (P>0.05)

3.3 Leaf chlorophyll content

Cytokinin application did not significantly affect the total leaf chlorophyll content in all the treatments compared with the controls. Nitrogen application showed no significant effect, either, on the chlorophyll content except in the absence of *Striga* (Figure 3.8)

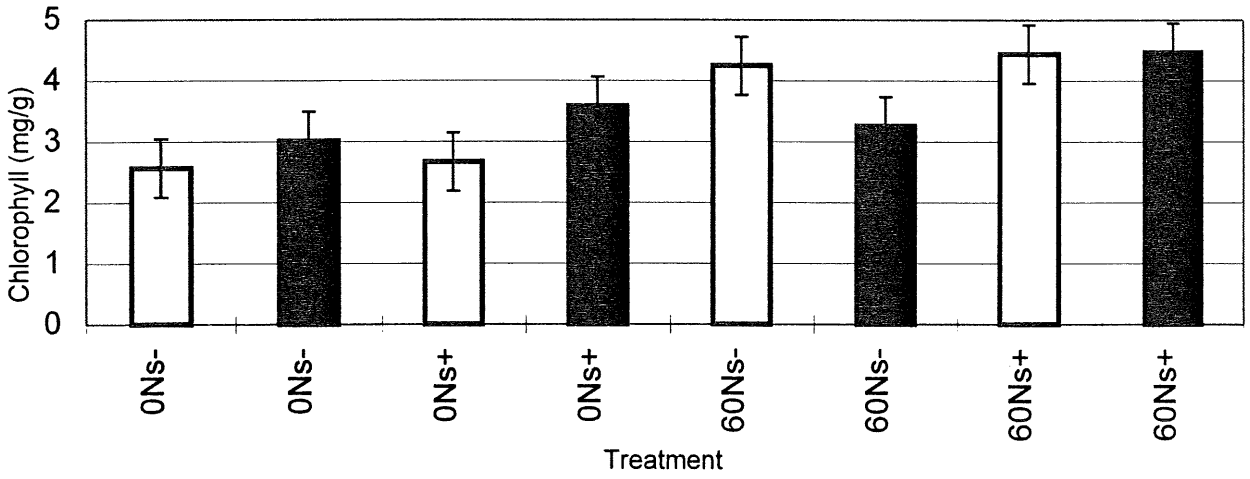

Figure 3.8 Effect of applied nitrogen, cytokinins and *Striga* on the chlorophyll content (mg/g) of sorghum leaf harvested 72 DAE. Only the main effect of nitrogen is significant (P = 0.05). See Figure 3.1 for details.

SPAD values, which correlate positively with the amount of chlorophyll in the leaf, did not show any statistical differences between the treatments throughout the time course of the measurements. Nitrogen application, however, resulted in a significant increase (P = 0.05) in SPAD values in the absence of *Striga*. (Appendix II)

3.4 Carboxylating enzymes and hormones

At the time of compilation of this report the results of the RUBISCO and PEP carboxylase and ABA/cytokinin analyses were pending. They could have helped in further explaining some of the observed effects of nitrogen and/or cytokinin applications on sorghum.

4. DISCUSSION

In this study, the initial light use efficiency decreased as a result of cytokinins (Figure 3.4). Amax increased for 0N, but decreased for 60N (Figure 3.6) This is consistent with chlorophyll content of the leaves (Figure 3.8).
Even though the activity of the carboxylating enzymes was not measured it is very unlikely that they could partly explain the observed effects especially in the presence of *Striga*. *Striga* has been reported to have little or no effect on the activity of the carboxylating enzymes in the host (Press and Cechin, 1994; Smith *et al.*, 1995). The increase in the rate of photosynthesis could be largely attributed to decrease in stomatal resistance observed in treatments receiving cytokinins especially in the absence of added nitrogen or in its presence together with *Striga* (Table 3.5). Low rates of photosynthesis observed in *Striga*-infected sorghum have been linked with stomatal limitations (Frost *et al.*, 1997). Cytokinins have been shown to limit stomatal closure in other plants including maize (Incoll and Whitelam, 1977; Blackman and Davies, 1983; Blackman and Davies, 1984; Incoll and Jewer, 1985). Transpiration and the flow of nutrients through the xylem are enhanced as a result of a decrease in stomatal resistance.

Despite the increase in photosynthetic rates, the allometry of the *Striga* infected plants was still greatly affected. Significantly less stem dry weight (Table 3.1), due to inhibition of internode expansion was found, whereas there was little or no negative effect on the leaf and root weight. This resulted in far less total biomass of the *Striga*-infected plants compared to the noninfected plants. This could be a result of loss of water, mineral nutrients and assimilates to *Striga*. *Striga* plants having relatively higher transpiration rates than their host (Press *et al.*, 1987b) deprive the host of a considerable amount of water. Although *Striga* is photosynthetic, ^{14}C-feeding studies and measurements of stable carbon isotopes demonstrate that the parasite is partially heterotrophic (Press *et al.*, 1987a). It still derives approximately 5-35% of carbon from the host (Cechin and Press, 1993a). The increase in root:shoot ratio (Table 3.1) of the *Striga*-infected sorghum could imply a greater amount of assimilates are used in maintenance respiration, thus less allowance for shoot growth.

Striga causes some significant damage while developing underground i.e. prior to the time of application of the exogenous hormones in this experiment. The fact that the effects of *Striga* on growth of sorghum were not reversed with increase in the photosynthesis rate suggests late time of application or some toxic effects. Nitrogen addition had some positive effect on the total biomass of the *Striga*-infected plants but not on the ligule height (Figure 3.2). Gurney *et al.*, 1995 also observed no effect of added nitrogen on the ligule height of sorghum and maize. The apparent increase in total biomass of *Striga*-infected plants with addition of nitrogen might be due to the significant delay ($P = 0.05$) in the time of emergence of *Striga* due to addition of nitrogen (Table 3.3). Even at a similar time of emergence of the first *Striga* shoot for the treatments with and without nitrogen application, the total biomass production in the former was increased (Figure

3.3). This again was another positive effect of nitrogen addition. *Striga* exerts much more severe effects on the host when it attaches to young plants. Delaying attachment reduced its deleterious effects on both host growth and photosynthesis (Press and Cechin, 1993; Cechin and Press, 1993c).

The interaction between applied nitrogen and cytokinins had a negative effect on photosynthesis in the noninfected plants (Table 3.4). This observation suggests that cytokinins might mediate some of the positive effects of applied nitrogen on growth and development of sorghum. Carbon allocation models have shown that nitrogen deprivation leads to reduced cytokinin production, a decreased rate of cytokinin export from the roots to the shoot, and decreased cytokinin concentrations (Van der Werf and Nagel, 1996). In the presence of *Striga*, application of cytokinins had a positive effect on sorghum photosynthesis irrespective of nitrogen. The *Striga* plants might have been acting as a sink for some of the nitrogen or synthesised cytokinins in sorghum thus keeping this hormone at a level well below the optimum. In that event exogenous cytokinins compensated for the endogenous cytokinins lost to the parasite. *Striga* depends upon the host for the cytokinins required for normal plumule development (Doggett, 1988). The good yield responses obtained by applying nitrogenous fertiliser suggest that competition for nitrogen is of major importance (Doggett, 1965; Drennan and El Hiweris, 1979; Ramaiah and Parker, 1982). It is also possible that because of *Striga* infection root uptake of nitrogen and its transport or that of cytokinins might have been perturbed with fewer amounts reaching the leaves. Applying exogenous cytokinins brought the level of total cytokinins in the leaf to optimum hence the observed positive effects.

Cytokinins appear to have an optimum concentration above which there is induction of negative effects of their direct or indirect functions in the plant. Similar (negative) effects have been observed in maize plant (Bassi *et al*, 1984) where RUBISCO and PEP carboxylase activities were stimulated by low and inhibited by high concentration of the cytokinin benzylaminopurine. The authors suggested that this hormone operates according to a stimulus-inhibition kinetic, the opposite morphogenic effects being produced by different concentrations. In wheat, cytokinin applications reduced chlorophyll content, photosynthesis and nitrogen content of leaves and grain at higher levels of added nitrogen (Herzog, 1981). Thus the observed decrease in photosynthesis could have been due to cytokinin-induced stomatal closure as a response to superoptimal concentration of the hormone. Stomatal conductance and assimilation were found to correlate positively for all treatments. Inhibition of activity of carboxylating enzymes could have as well been the cause for the decrease in rate of photosynthesis. Despite the measured decrease in assimilation rate, the total biomass at time of harvest showed a relative increase for the above treatment (Figure 3.1). An additional possible reason for the observed decrease in photosynthesis could have been a consequence of temporary sink limitation at the time of the measurement. The decline in the rate of net photosynthesis often observed in the afternoon is the

expression of a temporary sink limitation (Foyer, 1988), reflected in sugar and starch and starch accumulation, and either partial or total closure of the stomata (Hall and Milthorpe, 1978).

A decrease in the initial light use efficiency was also observed with cytokinin application (Figure 3.4). This decrease appeared to be more severe in the presence of *Striga*. Reductions in initial light use efficiency have been measured in *Striga*-infected sorghum plants when compared with the uninfected (Stewart *et al.*, 1991; Teklu, 1995; Ramlan and Graves, 1996). Decrease in photosynthetic efficiency has been linked to damage to photosystem II (PSII) (Kyle, 1987) and/or the dissipation of energy through photoprotective mechanisms (Demmig and Björkman, 1987; Henley *et al* 1991; Öquist *et al.*, 1992). It is thus possible that apart from the negative effects of applied cytokinins and nitrogen in the absence of *Striga* on the stomatal conductance, the activity of the carboxylating enzymes, and/or the amount of chlorophyll, this interaction might have rendered sorghum leaves more sensitive to photoinhibition due, probably, to damage to electron transport/photophosphorylation system. Another possible reason for the observed decrease in light use efficiency with cytokinin application could be the fact that more chlorophyll molecules were synthesised in the presence of cytokinin (Figure 3.8) with no accompanying increase in the reaction centres of photosynthesis. The chlorophyll content of the leaves of *Striga*-infected plants was higher than in the noninfected (Figure 3.8) in agreement with the findings of Frost *et al.* (1997). This could be the reason for the severe reduction in light use efficiencies in the presence of *Striga* and applied cytokinins as explained above.
There was an apparent increase in dark respiration in most treatments receiving cytokinins (Figure 3.7). This increase could have been because of the general increase in physiological activities, in particular photosynthesis, within the leaf because of cytokinin and/or nitrogen application. In general, increase in rate of photosynthesis is accompanied by increase in (maintenance) respiration. Some of the energy released during maintenance respiration is needed for the resynthesis of enzymes damaged during biochemical processes.
Energy is also needed to maintain the several concentration gradients in the plant which are diminished because of leakage of ions. This could partly explain the increase in respiration in the *Striga*-infected sorghum as ions are removed by *Striga*.
The trend of the effects of applied cytokinins and nitrogen on photosynthesis of *Striga*-infected plants at low light (Table 3.4) appears to contrast with the Amax values (Figure 3.6). A possible explanation for this observation is the fact that only two or three replicates were measured for the light response curves as opposed to five replicates for the A_{1535}. Some of the light response measurements, from which the Amax values are extrapolated, were done on different dates and distant from the glasshouse in contrast with A_{1535} measurements carried out on the same day and in the glasshouse under fairly similar conditions. Also, the observed effects of A_{1535} measurements are statiscally different ($P = 0.05$) as opposed to Amax values.

It is perhaps surprising that the increase in leaf photosynthesis due to cytokinin and nitrogen applications did not significantly affect the dry weights of sorghum infected with *Striga*. There may be at least three possible explanations for this observation. First, cytokinin application was only started when the infected plants were showing symptoms of chlorotic lesions typical of *Striga*-infected plants and after emergence of few *Striga* shoots. Because the pots were maintained in a well-watered state throughout the study, in order to avoid drought stress, appearance of chlorotic lesions due to *Striga* infection was somewhat delayed. The change in root:shoot balance and the resultant reduction in host shoot growth can occur at a very early stage of parasite growth, even before it emerges from the soil. In fact, Frost *et al.* (1997) observed changes in the height of the infected sorghum plants as early as 4 days after attachment before any measurable differences in photosynthesis could be determined. The timing of cytokinin application may have been rather late and that by this time there were already excessively high amounts of ABA whose effects could not be significantly reversed by the amount of exogenous cytokinins and added nitrogen. As a consequence, much of the assimilates were used, for example, in maintenance respiration at the expense of shoot growth. Elevated ABA amounts have been observed in *Striga*-infected plants and many of the symptoms observed suggested to be partly due to the effects of this hormone (Drennan & El Hiweris, 1979; Frost *et al.*, 1997; Taylor *et al.*, 1996). The impending determinations of the amount of ABA and cytokinins could further explain the observed effects.

Second, only the photosynthesis of some leaves was measured and not the canopy photosynthesis. It is not known whether the rate of photosynthesis in all the other leaves was also increased as a result of nitrogen and cytokinin applications. In fact, response of plants to applied cytokinins is known to vary with the age of the leaf or plant as a whole (Blackman and Davies, 1984; Incoll and Jewer, 1985), the stage of development of the plant (Caers and Vendrig, 1986) as well as on environmental conditions and genotype (Gianfagna, 1987). The leaves lower in the canopy may have been shielded from the in-coming radiation because of reduced internode expansion and, consequently, were not photosynthesising optimally resulting in an overall lower canopy photosynthesis of the infected plants.

Third, the rate of photosynthesis was estimated as the rate of CO_2 assimilation based on the changes in the CO_2 and water vapour content of the air passing through the leaf chamber. The assumption here is that the CO_2 taken up is involved in photosynthesis. There are other functions linked to CO_2 in the plant, for example enhanced uptake of minerals from the soil (Yalle *et al.*, 1987) or their shuttling from the roots to the shoot. The computed rate of photosynthesis might have been well above the actual rate in the leaves.

5. CONCLUSION

The results of this study indicate that *Striga* has an effect on sorghum hormone balance, and especially on the amount or activity of endogenous cytokinins. Nitrogen appears to have an effect on hormone balance within the *Striga**sorghum interaction as well as on the time of emergence of *Striga*. The effect(s) of *Striga* on hormone balance might be on the (bio)synthesis and/or transport and not on the interconversion into inactive forms of the already synthesised. Rapid inactivation of cytokinins is generally associated with application to intact plants (Tudor and Blakesley, 1986). The exogenous cytokinins were able to influence the photosynthesis of sorghum within the association which is an indication that they were active within the *Striga*-infected host.

There is also an indication from this study that increasing the rate of photosynthesis has little or no effect on the total biomass. This suggest that other physiological processes in sorghum, for example assimilate partitioning, are seriously affected with *Striga* infection.

Although in the study by Drennan and El Hiweris (1979) no significant effect was found after applying gibberellic acid (GA), the strong reduction in the internode expansion of the *Striga*-infected plants, even in the presence of added nitrogen or cytokinins, suggests that the role of GA might be an important one within this association. Neither the number of leaves nor root growth in the *Striga*-infected was significantly affected negatively. Similar effects have been observed in other plant systems after addition of plant growth retardants (Gianfagna, 1987). They (plant growth retardants) have little effect on the production of leaves or on root growth and act by inhibiting gibberellin biosynthesis. Their effects can be reversed by application of GA, but generally no other compounds are effective (Gianfagna, 1987). Further studies on the role of hormones in this association on a couple of cultivars are suggested. It would be interesting to observe the effect(s) of applying inhibitors of ABA synthesis or a combination of cytokinins and gibberellins with much emphasis on the time of application. Exceptionally high amounts of ABA have been correlated with most of the negative effects within the *Striga*-host association (Drennan & El Hiweris, 1979; Frost *et al.*, 1997; Taylor *et al.*, 1996). A more plausible approach would, however, be a manipulative using ABA-deficient mutants to understand mechanistically the regulation of plant growth under *Striga*-induced stress.

The possibility of a toxin is not ruled out given that in this study, increases in photosynthetic rates with cytokinin and/or nitrogen application did not result in corresponding increases of the total biomass in the *Striga* infected plants. The healthy roots in split-root experiments were unable to compensate for *Striga*-infected roots, even when the mass of the former exceeded that of the latter by a factor of three (Press and Stewart, 1987), unlike in the case of drought where the well-watered roots could compensate for droughted roots (Davies *et al.*, 1986; Schulze, 1986). Investigations on the nature of such a toxin are suggested. It is very likely that such a toxin could be interferring with the hormone balance given the severe effects of *Striga* on internode expansion.

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Appendix 1 Analyse grond binnengekomen: 9/4/97 - 5 m³

BEDRIJFSLABORATORIUM VOOR GROND- EN GEWASONDERZOEK
Oosterbeek

Postbus 115
6860 AC Oosterbeek

Meer informatie:
U kunt bellen: 026-3346440
of faxen: 026-3346419
Uw klantnummer is: 201.849.7

Bemestingsonderzoek

Bouwland

Unifarm
Haarwg 329
6709 RZ WAGENINGEN

Onderzoek

Datum verslag: 27-03-1997

Het verslag is geldig t/m 2000

Onderzoek-/ordernummer: 700107/000379385

Monster

Datum monstername: 26-02-1997

Perceel: A VOOR AAN DE WEG

Bemonsterde laag: 0 - 25 cm

Monster genomen door: BLGG

Grondsoort: Dekzand

Resultaat bepaald in droge grond volgens voorgeschreven methode

	Eenheid	Methode	Resultaat	Streef-niveau	Waardering
Fosfaat	mg P_2O_5/l	Pw	29	30 - 45	voldoende
Kali K-getal	mg K_2O/100 g	K-HCl	7 10	11 - 17	voldoende
Magnesia	mg MgO/kg	MgO-NaCl	137	75 - 174	ruim voldoende
Zuurgraad		pH-KCl	5,4	5,6	vrij laag
Organische stof	%	Gloeiverlies	4,7		

Advies in kg zuivere meststof per ha per jaar

	Frequentie	Gewas	Adviesgift	Afvoer
Fosfaat (P_2O_5)	per jaar	Consumptie-aardappelen Wintertarwe	140 100	55 75
Kali (K_2O)	per jaar	Consumptie-aardappelen Wintertarwe	270 135	255 110
Magnesium (MgO)	per jaar		1997 1998 1999 2000 0 0 65 65	
Kalk (zbw)	eenmalig		580 De kalkgift is gebaseerd op een bouwplan met 33 à 50 % aardappelen en 16 à 25 % suikerbieten.	

VF61-100.000-14-2-97-BLW

Appendix II Effect of nitrogen, cytokinin and *Striga hermonthica* on leaf chlorophyll content of sorghum measured with SPAD meter.